Shah 09/446,677 Page 1

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60 SEA FILE=HCAPLUS ("BIRKELUND S"/AU OR "BIRKELUND SVEN"/AU OR "BIRKELUND SVEND"/AU OR "BIRKELUND SVEND"/IN) 130 SEA FILE=HCAPLUS ("CHRISTIANSEN G"/AU OR "CHRISTIANSEN G"/IN L2 OR "CHRISTIANSEN G D"/AU OR "CHRISTIANSEN G G"/AU OR "CHRISTIAN SEN G R"/AU OR "CHRISTIANSEN G S"/AU) OR ("CHRISTIANSEN GUANNA"/AU OR "CHRISTIANSEN GUDRUN"/AU OR "CHRISTIANSEN GUNAA"/AU OR "CHRISTIANSEN GUNNA"/AU OR "CHRISTIANSEN GUNNA"/IN OR "CHRISTIANSEN GUNNAR"/AU) 139 SEA FILE=HCAPLUS PEDERSEN/AU OR ("PEDERSEN A"/AU OR "PEDERSEN L3A AA"/AU OR "PEDERSEN A G"/AU OR "PEDERSEN A G U"/AU OR "PEDERSEN A H"/AU OR "PEDERSEN A HJELHOLT"/AU OR "PEDERSEN A J"/AU OR "PEDERSEN A K"/AU OR "PEDERSEN A KIRSTEIN"/AU OR "PEDERSEN A L"/AU OR "PEDERSEN A M"/AU OR "PEDERSEN A M"/IN OR "PEDERSEN A MAGLE"/AU OR "PEDERSEN A N"/AU OR "PEDERSEN A O"/AU OR "PEDERSEN A R"/AU OR "PEDERSEN A S"/AU) 86 SEA FILE=HCAPLUS ("KNUDSEN K"/AU OR "KNUDSEN K"/IN OR "KNUDSEN L4K A"/AU OR "KNUDSEN K C"/AU OR "KNUDSEN K C B"/AU OR "KNUDSEN K C B"/IN OR "KNUDSEN K D"/AU OR "KNUDSEN K E"/AU OR "KNUDSEN K E B"/AU OR "KNUDSEN K E BACH"/AU OR "KNUDSEN K G"/AU OR "KNUDSEN K L"/AU OR "KNUDSEN K M"/AU OR "KNUDSEN K UHRE"/AU) OR ("KNUDSEN KATRINE"/AU OR "KNUDSEN KATRINE"/IN) 8 SEA FILE=HCAPLUS ("MYGIND P H"/AU OR "MYGIND PER"/AU OR L5 "MYGIND PER"/IN) 54 SEA FILE=HCAPLUS L1 AND L2 L6 O SEA FILE=HCAPLUS L3 AND L4 AND L5 AND L6 L7

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09/446,677 Page 2 Shah

=> d ibib abs hitrn 16 1-54

ANSWER 1 OF 54 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER:

2001:732281 HCAPLUS

TITLE:

Serological investigation of Mycoplasma genitalium in

infertile women

AUTHOR(S):

Clausen, Helle Friis; Fedder, Jens; Drasbek, Mette; Nielsen, Pernille K.; Toft, Bente; Ingerslev, Hans

Jakob; Birkelund, Svend; Christiansen,

.CORPORATE SOURCE:

Department of Medical Microbiology and Immunology, Department of Molecular and Structural Biology, University of Aarhus, Aarhus C, DK-8000, Den. Hum. Reprod. (2001), 16(9), 1866-1874 CODEN: HUREEE; ISSN: 0268-1161

. SOURCE:

PUBLISHER:

Oxford University Press

DOCUMENT TYPE:

Journal

LANGUAGE:

English

Background: The role of Mycoplasma genitalium in the pathogenesis of pelvic inflammatory disease has not been characterized. Methods: Sera from 308 infertile women were investigated for antibodies to M. genitalium by immunoblotting. Women with tubal factor infertility (TFI) made up 132 of the patients, 67 of the women had an infertile male partner and 109 were infertile for unknown reasons. Results: Of the TFI patients 29 (22.0%) were seropos. to the major adhesin, MgPa, of M. genitalium vs. 11 (6.3%) in the group of women with normal tubes. No cross-reactions between MgPa and P1 of the related Mycoplasma pneumoniae were found. Besides, MgPa pos. sera were confirmed by immunoblotting using a cloned fragment of the C-terminal part of MgPa specific to M. genitalium. Chlamydia trachomatis is known to be able to cause infertility as a result of salpingitis. Therefore, the sera were tested against C. trachomatis using a com. ELISA test. Seventy-five (56.8%) of the TFI patients were seropos. to C. trachomatis. Eight (27.6%) TFI patients seropos. to MgPa were neg. to C. trachomatis. Conclusions: This study indicates that M. genitalium may be an independent risk factor in the development of an inflammatory process leading to scarring of the uterine tubes in women and thereby causing infertility.

ANSWER 2 OF 54 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER:

2001:720238 HCAPLUS

TITLE:

Characterization of a hypervariable region in the

genome of Chlamydophila pneumoniae

AUTHOR(S):

Daugaard, L.; Christiansen, G.;

Birkelund, S.

CORPORATE SOURCE:

The Bartholin Building, Department of Medical Microbiology and Immunology, University of Aarhus,

DK-8000 C, Aarhus, Den.

SOURCE:

FEMS Microbiol. Lett. (2001), 203(2), 241-248

CODEN: FMLED7; ISSN: 0378-1097

PUBLISHER:

Elsevier Science B.V.

DOCUMENT TYPE:

Journal

LANGUAGE:

English

M. Smith 308-3278

Chlamydophila pneumoniae displays surprisingly little genomic variation, AB as seen by comparisons of the published genomes from three different isolates and sequencing of four different genes from different isolates. We have in the present study, however, demonstrated genomic variation between 10 C. pneumoniae isolates in the 11690-bp region between the two outer membrane protein genes pmp1 and pmp2. This region of the C. pneumoniae CWL-029 isolate contains seven C. pneumoniae-specific open reading frames (hb1-7, encoding hydrophobic beta-sheet-contg. proteins). We identified addnl. 12 open reading frames in the C. pneumoniae CWL-029 genome encoding hypothetical proteins with similarity to the seven hypothetical Hb-proteins. Compared to other isolates, genomic variation is seen to cause frame-shifting of three of the 19 hb-open reading frames, which are proposed to be three full-length genes and eight frame-shifted pseudogenes. The hypothetical proteins encoded by these proposed genes contain an N-terminally located highly hydrophobic stretch of 50-60 residues. A similar motif is found in all identified Chlamydia inclusion membrane proteins and therefore the Hb-proteins are candidate inclusion proteins.

ANSWER 3 OF 54 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER:

2001:720224 HCAPLUS

TITLE:

Differential expression of Pmp10 in cell culture

infected with Chlamydia pneumoniae CWL029

AUTHOR(S):

Pedersen, A. S.; Christiansen, G.;

Birkelund, S.

SOURCE:

FEMS Microbiol. Lett. (2001), 203(2), 153-159

CODEN: FMLED7; ISSN: 0378-1097

PUBLISHER:

Elsevier Science B.V.

DOCUMENT TYPE:

Journal

LANGUAGE:

English

The complete genome of Chlamydia pneumoniae contains a total of 21 genes encoding polymorphic membrane proteins (Pmp). From this large Pmp family three genes, pmp8, pmp10 and pmp11, were cloned and antibodies against recombinant full-length Pmp proteins were produced. Indirect immunofluorescence microscopy of HEp-2 cells infected with C. pneumoniae CWL029 was performed with the Pmp antibodies in combination with a Chlamydia-specific anti-lipopolysaccharide (LPS) antibody. This double staining technique clearly showed that expression of Pmp10 was differential. Addnl. double staining with monoclonal antibodies to the surface of C. pneumoniae elementary bodies and the anti-LPS antibody resulted in identification of seven monoclonal antibodies that reacted identically to the Pmp10 antibody indicating that Pmp10 is an immunodominant protein. Finally, the mol. mechanism responsible for differential expression is suggested to be variation in the guanine residues in the polyG tract of pmp10.

ANSWER 4 OF 54 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER:

2001:467428 HCAPLUS

TITLE:

Evaluation of real-time quantitative PCR for identification and quantification of Chlamydia pneumoniae by comparison with immunohistochemistry

AUTHOR(S):

Mygind, T.; Birkelund, S.; Falk, E.;

Christiansen, G.

CORPORATE SOURCE:

Department of Medical Microbiology and Immunology,

M. Smith 308-3278

Page 4 09/446,677 Shah

Aarhus, DK-8000, Den.

J. Microbiol. Methods (2001), 46(3), 241-251 SOURCE:

CODEN: JMIMDQ; ISSN: 0167-7012 Elsevier Science Ireland Ltd.

PUBLISHER:

Journal

DOCUMENT TYPE:

English LANGUAGE: AB

Chlamydia pneumoniae is a common cause of community-acquired pneumonia and it has been assocd. with atherosclerosis. C. pneumoniae has usually been diagnosed by serol. using a microimmunofluorescence test, but more recently polymerase chain reaction (PCR) has been viewed as an advantageous alternative. We developed a quant. real-time PCR for detection of C. pneumoniae. Primers were targeted for the pmp4 gene, and the PCR fragment was detected real-time with a fluorescence resonance energy transfer probe set using a LightCycler instrument. The PCR was used on DNA released from 50 .mu.m sections of paraffin-embedded formalin-fixed lung tissue from exptl. infected mice. Thereby, the no. of C. pneumoniae genomes was detd. To our knowledge this is the first time quantification of C. pneumoniae DNA has been attempted on paraffin-embedded formalin-fixed tissue. C. pneumoniae-specific immunohistochem. (IHC) was done on 5 .mu.m sections adjacent to the sections used in PCR, and the no. of inclusions were counted in each section. Good correlation was found when comparing results from PCR and IHC, which is in contrast to many previous studies.

REFERENCE COUNT: REFERENCE(S):

29

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- (2) Berger, M; J Lab Clin Med 2000, V136(3), P194 HCAPLUS
- (3) Boman, J; J Clin Microbiol 1999, V37(12), P3791 HCAPLUS
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- (9) Kalman, S; Nat Genet 1999, V21(4), P385 HCAPLUS ALL CITATIONS AVAILABLE IN THE RE FORMAT

ANSWER 5 OF 54 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER:

2001:456220 HCAPLUS

DOCUMENT NUMBER:

135:73609

TITLE:

Time-dependent expression and processing of a hypothetical protein of possible importance for regulation of the Chlamydia pneumoniae developmental

cycle

AUTHOR(S):

SOURCE:

Vandahl, Brian Berg; Gevaert, Kris; Demol, Hans; Hoorelbeke, Bart; Holm, Arne; Vandekerckhove, Joel;

Christiansen, Gunna; Birkelund, Svend

CORPORATE SOURCE:

Department of Medical Microbiology and Immunology,

University of Aarhus, Aarhus, DK-8000, Den. Electrophoresis (2001), 22(9), 1697-1704

CODEN: ELCTDN; ISSN: 0173-0835

PUBLISHER:

Wiley-VCH Verlag GmbH

DOCUMENT TYPE:

Journal English

LANGUAGE:

Chlamydia pneumoniae is an obligate intracellular human pathogen infecting epithelial cells of the upper respiratory tract. It is a Gram-neg.

bacteria and has a unique biphasic developmental cycle. In this study, we use two-dimensional gel electrophoresis in combination with radioactive labeling to investigate time-dependent expression and processing of C. pneumoniae proteins. We report on (i) the identification of a hypothetical protein which is expressed late in the developmental cycle and subsequently processed; we speculate that this protein may be of importance for the developmental cycle of Chlamydia; (ii) the identification of the major outer membrane protein in three different variants, which may all be present in vivo.

REFERENCE COUNT:

16

REFERENCE(S):

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- (2) Gevaert, K; Electrophoresis 1998, V19, P909 HCAPLUS
- (3) Grayston, J; J Infect Dis 2000, V181, PS402 HCAPLUS
- (4) Kalman, S; Nat Genet 1999, V21, P385 HCAPLUS
- (6) Mann, M; Biol Mass Spectrom 1993, V22, P338 HCAPLUS

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L6 ANSWER 6 OF 54 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER:

2001:371214 HCAPLUS

DOCUMENT NUMBER:

135:119326

TITLE:

Proteome analysis of the Chlamydia pneumoniae

elementary body

AUTHOR(S):

Vandahl, Brian Berg; Birkelund, Svend;

Demol, Hans; Hoorelbeke, Bart; Christiansen,

Gunna; Vandekerckhove, Joel; Gevaert, Kris

CORPORATE SOURCE:

Department of Medical Microbiology and Immunology,

University of Aarhus, Aarhus C, DK-8000, Den.

SOURCE: Electrophoresis (2001), 22(6), 1204-1223

CODEN: ELCTDN; ISSN: 0173-0835

PUBLISHER: Wiley-VCH Verlag GmbH DOCUMENT TYPE: Journal

DOCUMENT TYPE: LANGUAGE:

E: English

Chlamydia pneumoniae is an obligate intracellular human pathogen that AB causes acute and chronic respiratory tract diseases and that has been implicated as a possible risk factor in the development of atherosclerotic heart disease. C. pneumoniae cultivated in Hep-2 cells were 35S-labeled and infectious elementary bodies (EB) were purified. The EB proteins were sepd. by two-dimensional gel electrophoresis. Excised protein spots were in-gel digested with trypsin and peptides were concd. on reverse-phase chromatog. beads for identification anal. by matrix-assisted laser desorption/ionization-mass spectrometry. In the pH range from 3-11, 263 C. pneumoniae protein spots encoded from 167 genes were identified. These genes constitute 15% of the genome. The identified proteins include 31 hypothetical proteins. It has recently been suggested that EB should be able to synthesize ATP. This view may be strengthened by the identification of several proteins involved in energy metab. Furthermore, proteins have been found which are involved in the type III secretion app. important for pathogenesis of intracellular bacteria. Proteome maps and a table of all identified proteins have been made available on the world wide web at www.gram.au.dk.

Shah 09/446,677 Page 6

REFERENCE COUNT:

REFERENCE(S):

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(4) Bini, L; Electrophoresis 1996, V17, P185 HCAPLUS (5) Campbell, L; Infect Immun 1990, V58, P93 HCAPLUS _

(6) Christiansen, G; J Bacteriol 1993, V175, P1785

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ANSWER 7 OF 54 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER:

2000:589645 HCAPLUS

DOCUMENT NUMBER:

133:263634

TITLE:

Characterization of the variability of a 75-kDa

membrane protein in Mycoplasma hominis

AUTHOR(S):

Mygind, T.; Birkelund, S.;

CORPORATE SOURCE:

Dep. Med. Microbiol. Immunol., Bartholin Building,

Univ. Aarhus, Aarhus, DK-8000, Den.

SOURCE:

FEMS Microbiol. Lett. (2000), 190(1), 167-176

CODEN: FMLED7; ISSN: 0378-1097

PUBLISHER:

Elsevier Science B.V.

Christiansen, G.

DOCUMENT TYPE: LANGUAGE:

Journal English

The gene p75 encoding a 75-kDa surface-exposed membrane protein P75 was cloned and sequenced from Mycoplasma hominis type strain PG21T. To investigate the intraspecies variability, sequences were obtained from an addnl. two isolates 7488 and 183, and the three sequences were compared. The nucleotide and amino acid differences were not confined to specific regions of the gene/protein, but when comparing the three sequences, differences were present as single site substitutions or small insertions or deletions of nucleotides/amino acids. The intraspecies variability was further investigated by restriction enzyme anal. with two restriction enzymes (AluI and MboII) of PCR products amplified from p75 from 28 M. hominis isolates. On the basis of band patterns produced by the two restriction enzymes, the isolates could be divided into five and six groups. These groups neither matched categories of the M. hominis vaa gene nor the M. hominis p120 gene classes, indicating that the three genes vary by different mechanisms and possibly indicating horizontal gene

REFERENCE COUNT:

transfer.

28

REFERENCE(S):

(3) Boesen, T; Mol Microbiol 1998, V29, P97 HCAPLUS

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(5) Christiansen, G; Zbl Suppl 1990, V20, P535 HCAPLUS

(6) Devereux, J; Nucleic Acids Res 1984, V12, P387

HCAPLUS

(7) Fraser, C; Science 1995, V270, P397 HCAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

ANSWER 8 OF 54 HCAPLUS COPYRIGHT 2001 ACS 2000:511104 HCAPLUS

ACCESSION NUMBER: DOCUMENT NUMBER:

134:99305

TITLE:

Potential relevance of Chlamydia pneumoniae surface

proteins to an effective vaccine

AUTHOR(S):

Christiansen, Gunna; Pedersen, Anna-Sofie;

M. Smith 308-3278

09/446,677 Page 7 Shah

Hjerno, Karin; Vandahl, Brian; Birkelund,

Svend

Departments of Medical Microbiology and Immunology, CORPORATE SOURCE:

Aarhus, Den.

J. Infect. Dis. (2000), 181(Suppl. 3), S528-S537 SOURCE:

CODEN: JIDIAQ; ISSN: 0022-1899

PUBLISHER:

University of Chicago Press Journal

DOCUMENT TYPE: LANGUAGE:

English

The surface of Chlamydia pneumoniae is covered with proteins but their exact identification is not known probably because of the presence of conformational epitopes. A family of 21 pmp genes has been found by DNA sequencing. In common, these genes have the capacity to encode the amino acid motif GGAI. Several of the genes have the capacity to encode outer membrane proteins of about 100 kDa. Thus, they are candidate genes to encode the protein(s) present in the 98-kDa protein band of the C. pneumoniae outer membrane complex. The prodn. of recombinant GGAI proteins is described as is the use of polyclonal antibodies raised against the recombinant GGAI proteins to det. their expression in C. pneumoniae elementary bodies. At least three of the proteins, Omp4, 5, and 11, are expressed.

REFERENCE COUNT:

REFERENCE(S):

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- (5) Caldwell, H; Infect Immun 1981, V31, P1161 HCAPLUS
- (8) Christiansen, G; Am Heart J 1999, V138, PS491 **HCAPLUS**

ALL CITATIONS AVAILABLE IN THE RE FORMAT

ANSWER 9 OF 54 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER:

2000:454018 HCAPLUS

DOCUMENT NUMBER:

134:2362

TITLE:

SOURCE:

Genetic differences in the Chlamydia trachomatis tryptophan synthase .alpha.-subunit can explain

variations in serovar pathogenesis Shaw, Allan C.; Christiansen, Gunna;

AUTHOR(S): Roepstorff, Peter; Birkelund, Svend

Department of Medical Microbiology and Immunology, CORPORATE SOURCE:

University of Aarhus, Aarhus, DK-8000, Den. Microbes Infect. (2000), 2(6), 581-592

CODEN: MCINFS; ISSN: 1286-4579

Editions Scientifiques et Medicales Elsevier PUBLISHER:

Journal DOCUMENT TYPE: English LANGUAGE:

The human pathogen Chlamydia trachomatis is an obligate intracellular bacterium, characterized by a developmental cycle that alternates between the infectious, extracellular elementary bodies and intracellular, metabolically active reticulate bodies. The cellular immune effector interferon gamma (IFN-.gamma.) inhibits chlamydial multiplication in human epithelial cells by induction of the tryptophan degrading enzyme indoleamine 2,3-dioxygenase. IFN-.gamma. causes persistent C. trachomatis serovar A infections with atypical reticulate bodies that are unable to redifferentiate into elementary bodies and show diminished expression of important immunogens, but not of GroEL. However, the sensitivity to IFN-.gamma. varies among serovars of C. trachomatis. In our previous study significant IFN-.gamma.-specific, but tryptophan reversible, induction of proteins in C. trachomatis A and L2 with mol. masses of approx. 30 and 40 kDa was obsd. on 2D-gels. The 30-kDa protein from C. trachomatis L2 migrated with a significantly lower mol. wt. in C. trachomatis A. In this paper we include C. trachomatis B, C and D in our investigations and identify the proteins as alpha- and beta-subunits of the chlamydial tryptophan synthase using matrix-assisted laser desorption/ionization mass spectrometry. DNA sequencing of the trpA genes from C. trachomatis A and C shows that the TrpA in these serovars is a 7.7-kDa truncated version of C. trachomatis D and L2 TrpA. The truncation probably impairs the TrpA activity, thus elucidating a possible mol. mechanism behind variations in the pathogenesis of C. trachomatis serovars.

REFERENCE COUNT: REFERENCE(S):

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- (6) Bini, L; Electrophoresis 1996, V17, P185 HCAPLUS
- (7) Bjellqvist, B; Electrophoresis 1993, V14, P1023 HCAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

ANSWER 10 OF 54 HCAPLUS COPYRIGHT 2001 ACS L6

ACCESSION NUMBER:

2000:343954 HCAPLUS

DOCUMENT NUMBER:

133:348816

TITLE:

SOURCE:

Detection of Chlamydia trachomatis-specific antibodies in human sera by recombinant major outer-membrane

protein polyantigens

AUTHOR(S):

Mygind, Per; Christiansen, Gunna; Persson,

Kenneth; Birkelund, Svend

CORPORATE SOURCE:

Department of Medical Microbiology and Immunology,

University of Aarhus, Aarhus, DK-8000, Den. J. Med. Microbiol. (2000), 49(5), 457-465

CODEN: JMMIAV; ISSN: 0022-2615 Lippincott Williams & Wilkins

PUBLISHER: DOCUMENT TYPE:

LANGUAGE:

Journal English

This study was performed to generate and evaluate recombinant antigens for AB use in a species-specific C. trachomatis immunoassay. In a mol. genetic approach, fragments of the C. trachomatis major outer-membrane protein (MOMP) were produced as fusion proteins to create 3 different constructs encompassing the variable domains I, II and IV of selected C. trachomatis serovars. The recombinant MOMP polyantigens were affinity-purified and used in an ELISA. Antibody detection was evaluated with 103 patient sera and the results were compared with titers obtained in the micro-immunofluorescence test. The results showed that the generated MOMP

polyantigens detected the presence of C. trachomatis-specific human antibodies with little cross-reaction to C. pneumoniae-specific

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Shah

antibodies. When compared to the micro-immunofluorescence assay the MOMP polyantigen detected the presence of anti-C. trachomatis IgG antibodies with a sensitivity of 80% and a specificity of 91%.

REFERENCE COUNT:

REFERENCE(S):

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ANSWER 11 OF 54 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER:

2000:285351 HCAPLUS

DOCUMENT NUMBER:

133:71196

TITLE:

AUTHOR(S):

Membrane proteins PmpG and PmpH are major constituents of Chlamydia trachomatis L2 outer membrane complex

Mygind, P. H.; Christiansen, G.; Roepstorff,

P.; Birkelund, S.

CORPORATE SOURCE:

Department of Medical Microbiology and Immunology,

University of Aarhus, Aarhus, DK-8000, Den. FEMS Microbiol. Lett. (2000), 186(2), 163-169

CODEN: FMLED7; ISSN: 0378-1097

SOURCE:

Elsevier Science B.V.

DOCUMENT TYPE:

Journal

PUBLISHER: LANGUAGE:

English

The outer membrane complex of Chlamydia is involved in the initial adherence and ingestion of Chlamydia by the host cell. In order to identify novel proteins in the outer membrane of Chlamydia trachomatis L2, proteins were sepd. by sodium dodecyl sulfate polyacrylamide gel electrophoresis. By silver staining of the protein profile, a major protein doublet of 100-110 kDa was detected. In-gel tryptic digestion and matrix-assisted laser desorption/ionization mass spectrometry identified these proteins as the putative outer membrane proteins PmpG and PmpH.

REFERENCE COUNT:

22

REFERENCE(S):

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- (3) Buendia, A; FEMS Microbiol Lett 1997, V150, P113 **HCAPLUS**
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ALL CITATIONS AVAILABLE IN THE RE FORMAT

HCAPLUS COPYRIGHT 2001 ACS ANSWER 12 OF 54

ACCESSION NUMBER:

2000:60728 HCAPLUS

DOCUMENT NUMBER:

132:304112

TITLE:

Cloning, sequencing and variability analysis of the

gap gene from Mycoplasma hominis

Mygind, T.; Zeuthen Sogaard, I.; Melkova, R.; Boesen,

T.; Birkelund, S.; Christiansen, G.

M. Smith 308-3278

AUTHOR(S):

Page 10 09/446,677 Shah

CORPORATE SOURCE:

Department of Medical Microbiology and Immunology,

University of Aarhus, Aarhus, DK-8000, Den. FEMS Microbiol. Lett. (2000), 183(1), 15-21

CODEN: FMLED7; ISSN: 0378-1097

PUBLISHER:

SOURCE:

Elsevier Science B.V.

DOCUMENT TYPE:

Journal

LANGUAGE:

English

The gap gene encodes the glycolytic enzyme glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The gene was cloned and sequenced from the Mycoplasma hominis type strain PG21T. The intraspecies variability was investigated by inspection of restriction fragment length polymorphism (RFLP) patterns after polymerase chain reaction (PCR) amplification of the gap gene from 15 strains and furthermore by sequencing of part of the gene in eight strains. The M. hominis gap gene was found to vary more than the Escherichia coli counterpart, but the variation at nucleotide level gave rise to only a few amino acid substitutions. To verify that the gene was expressed in M. hominis, a polyclonal antibody was produced and tested against whole cell protein from 15 strains. The enzyme was expressed in all strains investigated as a 36-kDa protein. All strains except type strain PG21T showed reaction to a 104-kDa band in addn. to the expected 36-kDa band. The protein reacting at 104 kDa is a M. hominis protein with either an epitope similar to one on GAPDH, or it is an Ig binding protein.

REFERENCE COUNT: REFERENCE(S):

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(4) Christiansen, G; Int J Syst Bacteriol 1988, V38, P108 HCAPLUS

(5) Fraser, C; Science 1995, V270, P397 HCAPLUS

(6) Grau, O; Int J Syst Bacteriol 1991, V41, P473 HCAPLUS

(7) Guttman, D; Genetics 1994, V138, P993 HCAPLUS

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HCAPLUS COPYRIGHT 2001 ACS ANSWER 13 OF 54

25

ACCESSION NUMBER:

1999:764647 HCAPLUS

DOCUMENT NUMBER:

132:206663

TITLE:

AUTHOR(S):

Molecular biology of Chlamydia pneumoniae surface proteins and their role in immunopathogenicity

Christiansen, Gunna; Boesen, Thomas; Hjerno,

Karin; Daugaard, Lene; Mygind, Per; Madsen, Anna Sofie; Knudsen, Katrine; Falk, Erling; Birkelund,

CORPORATE SOURCE:

Department of Medical Microbiology and Immunology and

the Department of Molecular and Structural Biology,

University of Aarhus, Aarhus, DK-8000, Den. Am. Heart J. (1999), 138(5, Pt. 2), S491-S495

CODEN: AHJOA2; ISSN: 0002-8703

PUBLISHER:

Mosby, Inc.

DOCUMENT TYPE:

Journal

SOURCE:

English LANGUAGE:

Background. The assocn. of Chlamydia pneumoniae with the development of atherosclerosis is based on serol. and on detection of C pneumoniae-specific DNA by polymerase chain reaction in the atheromas. Methods and Results. Because the humoral immune response frequently

recognizes epitopes present on the surface of the bacteria, we analyzed what components are present on the C. pneumoniae surface. We identified a family of proteins, the GGAl or Omp4-15 proteins, of which at least 3 are present on the surface of C. pneumoniae. We immunized rabbits with recombinant GGAl proteins and used these antibodies in immunofluorescence microscopy of exptl. infected mice. In lung sections, a massive infiltration with polymorph nuclear neutrophil cells was obsd. In the bronchial epithelial cells, C. pneumoniae inclusions were seen. Evidence was found of differential expression of the GGAl proteins. Conclusions. On the basis of surface localization, differential expression, and the fact that the proteins are recognized by the human humoral immune response, we speculate whether these proteins, in addn. to the lipopolysaccharides, are of importance for the immunopathogenesis of C

REFERENCE COUNT:

REFERENCE(S):

13

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- (4) Dhir, S; J Immunol 1972, V109, P116 HCAPLUS
- (6) Klemm, P; Mol Microbiol 1990, V4, P553 HCAPLUS
- (8) Longbottom, D; FEMS Microbiol Lett 1998, V164, P111 HCAPLUS
- (13) Stephens, R; Science 1998, V282, P754 HCAPLUS ALL CITATIONS AVAILABLE IN THE RE FORMAT

ANSWER 14 OF 54 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER:

1999:342767 HCAPLUS

DOCUMENT NUMBER:

131:42382

TITLE:

Mapping and identification of interferon

gamma-regulated HeLa cell proteins separated by

immobilized pH gradient 2-dimensional gel

electrophoresis

AUTHOR(S):

Shaw, Allan Christian; Rossel Larsen, Martin; Roepstorff, Peter; Justesen, Just; Christiansen,

Gunna; Birkelund, Svend

CORPORATE SOURCE:

Department Medical Microbiology Immunology, University

SOURCE:

Aarhus, Aarhus, DK-8000, Den. Electrophoresis (1999), 20(4-5), 984-993

CODEN: ELCTDN; ISSN: 0173-0835

PUBLISHER:

LANGUAGE:

Wiley-VCH Verlag GmbH

DOCUMENT TYPE:

Journal English

Interferon .gamma. (IFN-.gamma.) is a potent immunomodulatory lymphokine, AB secreted by activated T-lymphocytes and NK-cells during the cellular immune response. Actions of IFN-.gamma. are mediated through binding to the IFN-.gamma.-receptor, present on most cells, and the subsequent activation of a great magnitude of IFN-.gamma. responsive genes was reported previously. IFN-.gamma.-regulated HeLa cell proteins were identified and mapped dy 2-D PAGE with the immobilized pH gradient (IPG) 2-D PAGE system. A semiconfluent layer of HeLa cells was grown on tissue culture plates, and changes in protein expression due to 100 U/mL IFN-.gamma. were investigated at different periods after treatment, using pulse labeling with [35S]Met/Cys in combination with 2-D PAGE (IPG). The identity of 8 protein spots was elucidated by matrix-assisted laser desorption/ionization-mass spectrometry (MALDI-MS), and several variants

of the IFN-.gamma.-inducible tryptophanyl-tRNA synthetase (hWRS) were detected by immunoblotting.

REFERENCE COUNT:

REFERENCE(S):

- (1) Aki, M; J Biochem 1994, V115, P257 HCAPLUS
- (2) Beresini, M; Electrophoresis 1990, V11, P232 **HCAPLUS**
- (3) Boehm, U; Annu Rev Immunol 1997, V15, P749 HCAPLUS
- (4) Celis, J; Leukemia 1987, V1, P800 HCAPLUS
- (6) Chou, Y; Proc Natl Acad Sci USA 1989, V86, P1885 **HCAPLUS**

ALL CITATIONS AVAILABLE IN THE RE FORMAT

ANSWER 15 OF 54 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER:

1999:342766 HCAPLUS

DOCUMENT NUMBER:

131:197411

TITLE:

Mapping and identification of HeLa cell proteins separated by immobilized pH-gradient two-dimensional

gel electrophoresis and construction of a

two-dimensional polyacrylamide gel electrophoresis

AUTHOR(S):

Shaw, Allan Christian; Rossel Larsen, Martin; Roepstorff, Peter; Holm, Arne; Christiansen,

Gunna; Birkelund, Svend

CORPORATE SOURCE:

Department Medical Microbiology Immunology, University

Aarhus, Aarhus, DK-8000, Den.

SOURCE:

Electrophoresis (1999), 20(4-5), 977-983

CODEN: ELCTDN; ISSN: 0173-0835

PUBLISHER:

Wiley-VCH Verlag GmbH

DOCUMENT TYPE:

Journal

LANGUAGE:

English

The HeLa cell line, a human adenocarcinoma, is used in many research AΒ fields, since it can be infected with a wide range of viruses and intracellular bacteria. The mapping of HeLa cell proteins is useful for the investigation of parasite host cell interactions. Because of the recent improvements of 2-D gel electrophoresis with immobilized pH gradients (IPG) compared to isoelec. focusing with carrier ampholytes, a highly reproducible method for examg. global changes in HeLa cell protein expression due to different stimuli is now available. The authors have initiated the mapping of [35S]Met/Cys-labeled HeLa cell proteins with the 2-D PAGE (IPG)-system, using matrix-assisted laser desorption/ionizationmass spectrometry (MALDI-MS) and N-terminal sequencing for protein identification. To date 21 proteins were identified and mapped. To make these and future data accessible for interlab. comparison, the authors constructed a 2-D PAGE database on the World Wide Web.

REFERENCE COUNT:

REFERENCE(S):

- (1) Appel, R; Electrophoresis 1996, V17, P540 HCAPLUS
- (2) Birkelund, S; Electrophoresis 1997, V18, P563 **HCAPLUS**
- (3) Bjellqvist, B; Electrophoresis 1993, V14, P1023 **HCAPLUS**
- (4) Bjellqvist, B; Electrophoresis 1994, V15, P529 **HCAPLUS**
- (5) Blomberg, A; Electrophoresis 1995, V16, P1935 HCAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

HCAPLUS COPYRIGHT 2001 ACS ANSWER 16 OF 54

ACCESSION NUMBER:

1999:342738 HCAPLUS

DOCUMENT NUMBER:

131:72576

TITLE:

Effects of interferon gamma on Chlamydia trachomatis serovar A and L2 protein expression investigated by

two-dimensional gel electrophoresis

AUTHOR(S):

Shaw, Allan Christian; Christiansen, Gunna;

Birkelund, Svend

CORPORATE SOURCE:

Department Medical Microbiology Immunology, University

Aarhus, Aarhus, DK-8000, Den.

SOURCE:

Electrophoresis (1999), 20(4-5), 775-780

CODEN: ELCTDN; ISSN: 0173-0835

PUBLISHER:

Wiley-VCH Verlag GmbH Journal

DOCUMENT TYPE:

English

LANGUAGE: C. trachomatis is an obligate intracellular bacterium causing human ocular and genital disease. The lymphokine interferon .gamma. (IFN-.gamma.) is an important immune effector exerting antimicrobial effects towards several intracellular parasites, the chlamydia included. IFN-.gamma. was reported to inhibit the chlamydial replication in vitro in part by depleting intracellular levels of tryptophan in a dose-dependent manner. Down-regulation of important immunogens was described. These findings are extended here, and the authors combined pulse labeling with [35S]Met and 2-D gel electrophoresis with immobilized pH gradients to investigate changes in the protein expression of C. trachomatis serovar A and L2 caused by treatment with IFN-.gamma.. In contrast to what was obsd. in C. trachomatis L2, the results showed that, in C. trachomatis A, down-regulations of the chlamydia major outer membrane protein and other proteins were detectable upon IFN-.gamma. treatment. The authors report the up-regulations of C. trachomatis A and L2 proteins with mol. masses of 30 kDa and 40 kDa which may be part of an, as yet, uncharacterized chlamydial response to IFN-.gamma. treatment.

REFERENCE COUNT:

25

REFERENCE(S):

(2) Beatty, W; Infect Immun 1995, V63, P199 HCAPLUS

(3) Beatty, W; Proc Natl Acad Sci USA 1993, V90, P3998 HCAPLUS

(4) Bini, L; Electrophoresis 1996, V17, P185 HCAPLUS

(5) Bjellqvist, B; Electrophoresis 1993, V14, P1023 **HCAPLUS**

(6) Byrne, G; Infect Immun 1986, V53, P347 HCAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

ANSWER 17 OF 54 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: DOCUMENT NUMBER:

1999:27851 HCAPLUS 130:92748

TITLE:

Outer membrane proteins of Chlamydia pneumoniae and the genes encoding them and their diagnostic and

therapeutic uses

INVENTOR(S):

Birkelund, Svend; Christiansen, Gunna;

Knudsen, Katrine; Madsen, Anna-Sofie; Mygind, Per

PATENT ASSIGNEE(S):

SOURCE:

PCT Int. Appl., 115 pp.

M. Smith 308-3278

CODEN: PIXXD2

DOCUMENT TYPE:

Patent English

LANGUAGE:

FAMILY ACC. NUM. COUNT:

PATENT INFORMATION:

APPLICATION NO. DATE DATE KIND PATENT NO. ____ WO 1998-DK266 19980619 19981230 A2 WO 9858953 19990318 A3 WO 9858953 W: AL, AM, AT, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, CZ, DE, DE, DK, DK, EE, EE, ES, FI, FI, GB, GE, GH, GM, GW, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM RW: GH, GM, KE, LS, MW, SD, SZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, ML, MR, NE, SN, TD, TG 19980619 AU 1998-80119 19990104 A1 AU 9880119 EP 1998-928179 19980619 20000614 EP 1007685 A2 R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI 19980619 20000919 BR 1998-10288 BR 9810288

PRIORITY APPLN. INFO.:

DK 1997-744

A 19970623

WO 1998-DK266

W 19980619

AB Members of a gene family from the human respiratory pathogen Chlamydia pneumoniae that encode surface exposed membrane proteins of a size of

pneumoniae that encode surface exposed membrane proteins of a size of approx. 89-101 kDa and of 56-57 kDa, preferably about 89.6-100.3 kDa and about 56.1 kDa are cloned and characterized. The genes and gene products can be used in the diagnosis, pathol. and epidemiol. of C. pneumoniae and in vaccines. Genes were cloned by screening an expression library with antiserum to Chlamydia outer membrane complexes.

L6 ANSWER 18 OF 54 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1999:25056 HCAPLUS

DOCUMENT NUMBER: 130:218977

TITLE: Identification of two novel genes encoding 97- to

99-kilodalton outer membrane proteins of Chlamydia

pneumoniae

AUTHOR(S): Knudsen, Katrine; Madsen, Anna Sofie; Mygind, Per;

Christiansen, Gunna; Birkelund, Svend

CORPORATE SOURCE: Department of Medical Microbiology and Immunology,

University of Aarhus, Aarhus, DK-8000, Den.

SOURCE: Infect. Immun. (1999), 67(1), 375-383

CODEN: INFIBR; ISSN: 0019-9567

American Society for Microbiology

DOCUMENT TYPE: Journal LANGUAGE: English

AB Two genes encoding 97- to 99-kDa Chlamydia pneumoniae VR1310 outer membrane proteins (Omp4 and Omp5) with mutual similarity were cloned and sequenced. The proteins were shown to be constituents of the C. pneumoniae outer membrane complex, and the deduced amino acid sequences were similar to those of putative outer membrane proteins encoded by the

PUBLISHER:

Chlamydia psittaci and Chlamydia trachomatis gene families. By use of a monospecific polyclonal antibody against purified recombinant Omp4, it was shown that without heating, the protein migrated at 65 to 75 kDa in sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Immunoelectron microscopy showed that epitopes of Omp4 were exposed on the surface of C. pneumoniae elementary bodies, reticulate bodies, and outer membrane complex. Proteins encoded by the C. pneumoniae gene family seem to be dominant antigens in exptl. infected mice.

REFERENCE COUNT:

REFERENCE(S):

41

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- (2) Bhat, K; Mol Microbiol 1991, V5, P1889 HCAPLUS
- (3) Birkelund, S; Infect Immun 1989, V57, P2683 HCAPLUS
- (5) Buendia, A; FEMS Microbiol Lett 1997, V150, P113 HCAPLUS
- (6) Caldwell, H; Infect Immun 1981, V31, P1161 HCAPLUS ALL CITATIONS AVAILABLE IN THE RE FORMAT

HCAPLUS COPYRIGHT 2001 ACS ANSWER 19 OF 54 1.6

ACCESSION NUMBER:

1998:722395 HCAPLUS

DOCUMENT NUMBER:

130:63445

TITLE:

Topological analysis of Chlamydia trachomatis L2 outer

membrane protein 2

AUTHOR(S):

SOURCE:

Mygind, Per; Christiansen, Gunna;

Birkelund, Svend

CORPORATE SOURCE:

Department of Medical Microbiology and Immunology,

University of Aarhus, Aarhus C, DK-8000, Den.

J. Bacteriol. (1998), 180(21), 5784-5787
CODEN: JOBAAY; ISSN: 0021-9193

PUBLISHER:

American Society for Microbiology

DOCUMENT TYPE:

Journal

LANGUAGE: .

English

Using monospecific polyclonal antisera to different parts of Chlamydia trachomatis L2 outer membrane protein 2 (Omp2), we show that the protein is localized at the inner surface of the outer membrane. Omp2 becomes immunoaccessible when Chlamydia elementary bodies are treated with dithiothreitol, and protease digestions indicate that Omp2 has a possible two-domain structure.

REFERENCE COUNT:

REFERENCE(S):

- (1) Allen, J; Mol Microbiol 1990, V4, P1543 HCAPLUS
- (2) Bavoil, P; Infect Immun 1984, V44, P479 HCAPLUS
- (4) Birkelund, S; Infect Immun 1988, V56, P654 HCAPLUS
- (7) Caldwell, H; Infect Immun 1981, V31, P1161 HCAPLUS
- (8) Collett, B; J Gen Microbiol 1989, V135, P85

HCAPLUS

ALL CITATIONS AVAILABLE IN THE RE FORMAT

HCAPLUS COPYRIGHT 2001 ACS ANSWER 20 OF 54

ACCESSION NUMBER:

1998:620358 HCAPLUS

DOCUMENT NUMBER:

130:11161

TITLE:

DNA sequencing reveals limited heterogeneity in the

16S rRNA gene from the rrnB operon among five

Mycoplasma hominis isolates

Mygind, Tina; Birkelund, Svend; AUTHOR(S):

09/446,677 Page 16 Shah

Christiansen, Gunna

CORPORATE SOURCE:

Department of Medical Microbiology and Immunology,

University of Aarhus, Aarhus, DK-8000, Den.

Int. J. Syst. Bacteriol. (1998), 48(3), 1067-1071 SOURCE:

CODEN: IJSBA8; ISSN: 0020-7713 Society for General Microbiology

PUBLISHER:

Journal English

DOCUMENT TYPE: LANGUAGE:

To investigate the intraspecies heterogeneity within the 16S rRNA gene of AB Mycoplasma hominis, five isolates with diverse antigenic profiles, variable/identical P120 hypervariable domains, and different 16S rRNA gene RFLP patterns were analyzed. The 16S rRNA gene from the rrnB operon was amplified by PCR and the PCR products were sequenced. Three isolates had identical 16S rRNA sequences and two isolates had sequences that differed from the others by only one nucleotide.

REFERENCE COUNT: REFERENCE(S):

- (1) Amikam, D; J Bacteriol 1984, V158, P376 HCAPLUS
- (2) Blanchard, A; J Clin Microbiol 1993, V31, P1358 **HCAPLUS**
- (3) Christiansen, G; Int J Syst Bacteriol 1988, V38, P108 HCAPLUS
- (4) Christiansen, G; Zentbl Bakteriol Suppl 1990, V20, P535 HCAPLUS
- (5) Grau, O; Int J Syst Bacteriol 1991, V41, P473 **HCAPLUS**

ALL CITATIONS AVAILABLE IN THE RE FORMAT

ANSWER 21 OF 54 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER:

1998:547882 HCAPLUS

DOCUMENT NUMBER:

129:287693

TITLE:

Antigenic and genomic homogeneity of successive

Mycoplasma hominis isolates

AUTHOR(S):

SOURCE:

Jensen, Lise T.; Thorsen, P.; Moller, B.;

Birkelund, S.; Christiansen, G.

CORPORATE SOURCE:

Department of Medical Microbiology and Immunology,

University of Aarhus, Aarhus C, DK-8000, Den. J. Med. Microbiol. (1998), 47(8), 659-666

CODEN: JMMIAV; ISSN: 0022-2615

PUBLISHER:

Lippincott-Raven Publishers

DOCUMENT TYPE:

LANGUAGE:

Journal English

Sixty M. hominis isolates were obtained from the cervices of pregnant women and from the ears or pharynges of their newborn babies. The isolates were examd. by SDS-PAGE and pulsed-field gel electrophoresis. Antigenic and genomic profiles were obtained for 16 series with 2 or more successive isolates. Both analyses led to the conclusion that isolates from the same woman were identical or nearly identical, while isolates from different women exhibited a high degree of variation with respect to both genomic and antigenic profiles.

ANSWER 22 OF 54 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER:

1998:489146 HCAPLUS

DOCUMENT NUMBER:

129:212306

TITLE:

The Mycoplasma hominis vaa gene displays a mosaic gene

09/446,677 . Page 17 Shah

structure

Boesen, Thomas; Emmersen, Jeppe; Jensen, Lise T.; AUTHOR(S):

Ladefoged, Soren A.; Thorsen, Poul; Birkelund,

Svend; Christiansen, Gunna

Department of Medical Microbiology and Immunology, The CORPORATE SOURCE:

Bartholin Building, University of Aarhus, Aarhus C,

DK-8000, Den.

Mol. Microbiol. (1998), 29(1), 97-110 SOURCE:

CODEN: MOMIEE; ISSN: 0950-382X

Blackwell Science Ltd. PUBLISHER:

Journal DOCUMENT TYPE: English LANGUAGE:

Mycoplasma hominis contains a variable adherence-assocd. (vaa) gene. classify variants of the vaa genes, we examd. 42 M. hominis isolates by PCR, DNA sequencing and immunoblotting. This uncovered the existence of five gene categories. Comparison of the gene types revealed a modular compn. of the Vaa proteins. The proteins constituted a conserved N-terminal part followed by a varying no. of interchangeable cassettes encoding approx. 110 amino acids with conserved sequence boxes flanking the cassettes. The interchangeable cassettes showed a high mutual homol. and a conserved leucine zipper motif. The smallest product contained only one cassette and the largest five. Addnl., two types of stop mutations caused by substitutions resulting in the expression of truncated Vaa proteins were obsd. Our results expand the known potential of the Vaa system in generating antigen variation.

ANSWER 23 OF 54 HCAPLUS COPYRIGHT 2001 ACS

1998:469401 HCAPLUS ACCESSION NUMBER:

DOCUMENT NUMBER:

129:243752

Transmission electron microscopy and immunogold TITLE:

staining of mollicute surface antigens

Christiansen, Gunna; Birkelund, AUTHOR(S):

Svend

Department of Medical Microbiology and Immunology, CORPORATE SOURCE:

University of Aarhus, Den.

Methods Mol. Biol. (Totowa, N. J.) (1998), SOURCE:

104 (Mycoplasma Protocols), 309-318 CODEN: MMBIED; ISSN: 1064-3745

Humana Press Inc. PUBLISHER:

Journal DOCUMENT TYPE: English LANGUAGE:

Mollicutes are cell wall-less bacteria. The mollicute cell membrane contains essentially all the cellular lipids and a substantial fraction of the cellular proteins; the molar ratio of lipid-to-protein is approx. 60:1. Both neg. staining and immunogold labeling of mollicutes are described here; Mycoplasma hominis is used as a specific example. Growth media for cultivation of the microorganisms; neg. staining with PTA (phosphotungstic acid) followed by electron microscopy; and immunogold staining using primary and secondary antibodies and colloidal gold, followed by electron microscopy, are described.

ANSWER 24 OF 54 HCAPLUS COPYRIGHT 2001 ACS

1998:328974 HCAPLUS ACCESSION NUMBER:

129:80346 DOCUMENT NUMBER:

Shah 09/446,677 Page 18

TITLE: Analysis of the humoral immune response to Chlamydia

outer membrane protein 2

AUTHOR(S): Mygind, Per; Christiansen, Gunna; Persson,

Kenneth; Birkelund, Svend

CORPORATE SOURCE: Department of Medical Microbiology and Immunology,

University of Aarhus, Aarhus, DK-8000, Den.

Clin. Diagn. Lab. Immunol. (1998), 5(3), 313-318

CODEN: CDIMEN; ISSN: 1071-412X
American Society for Microbiology

PUBLISHER: America:
DOCUMENT TYPE: Journal
LANGUAGE: English

SOURCE:

LANGUAGE:

English

The humoral immune response to Chlamydia outer membrane protein 2 (Omp2) was studied. Omp2 is a highly genus-conserved structural protein of all Chlamydia species, contg. a variable N-terminal fragment. To analyze where the immunogenic parts were localized, seven highly purified truncated fusion proteins constituting different regions of the protein were produced (Chlamydia pneumoniae-Ompaa23-aa93, Chlamydia psittaci-Omp2aa23-aa94, and Chlamydia trachomatis-Omp2aa23-aa84, aa87-aa547, aa23-aa182, aa167-aa434, aa420-aa547). By an ELISA with serol. defined patient sera, Omp2 was a major immunogen of both C. pneumoniae and C. trachomatis infections (P.mchlt. 0.0001). The humoral immune responses were not confined to any particular region of the Omp2 protein, and no species-specific anti-Omp2 Igs were detected.

L6 ANSWER 25 OF 54 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1997:583225 HCAPLUS

DOCUMENT NUMBER: 127:261105

TITLE: Chlamydia trachomatis utilizes the host cell

microtubule network during early events of infection

AUTHOR(S): Clausen, Johannes D.; Christiansen, Gunna;

Holst, Henrik U.; Birkelund, Svend

CORPORATE SOURCE: Departments of Medical Microbiology and Immunology,

University of Aarhus, Aarhus C, DK-8000, Den.

SOURCE: Mol. Microbiol. (1997), 25(3), 441-449

CODEN: MOMIEE; ISSN: 0950-382X

PUBLISHER: Blackwell DOCUMENT TYPE: Journal LANGUAGE: English

LANGUAGE: The host cell cytoskeleton is known to play a vital role in the life cycles of several pathogenic intracellular microorganisms by providing the basis for a successful invasion and by promoting movement of the pathogen once inside the host cell cytoplasm. McCoy cells infected with Chlamydia trachomatis serovars E or L2 revealed, by indirect immunofluorescence microscopy, co-location of microtubules and Chlamydia-contg. vesicles during the process of migration from the host cell surface to a perinuclear location. The vast majority of microtubule-assocd. Chlamydia vesicles also co-located with tyrosine-phosphorylated McCoy cell proteins. After migration, the Chlamydia-contg. vesicles were positioned exactly at the center of the microtubule network, indicating a microtubule-dependent mode of chlamydial redistribution. Inhibition of host cell dynein, a microtubule-dependent motor protein known to be involved in directed vesicle transport along microtubules, was obsd. to have a pronounced effect on C. trachomatis infectivity. Furthermore, dynein was found to co-locate with perinuclear aggregates of C. trachomatis E and L2 but not

C. pneumoniae VR-1310, indicating a marked difference in the cytoskeletal requirements for C. trachomatis and C. pneumoniae during early infection In support of this view, C. pneumoniae VR-1310 was shown to induce much less tyrosine phosphorylation of HeLa cell proteins during uptake than that seen for C. trachomatis.

HCAPLUS COPYRIGHT 2001 ACS ANSWER 26 OF 54

ACCESSION NUMBER:

1997:336552 HCAPLUS

DOCUMENT NUMBER:

127:62746

TITLE:

AUTHOR(S):

Characterization of Chlamydia trachomatis L2-induced

tyrosine-phosphorylated HeLa cell proteins by

two-dimensional gel electrophoresis

Birkelund, Svend; Bini, Luca; Pallini,

Vitaliano; Sanchez-Campillo, Maria; Liberatori, Sabrina; Clausen, Johannes D.; Ostergaard, Soren;

Holm, Arne; Christiansen, Gunna

CORPORATE SOURCE:

Department Medical Microbiology Immunology, University

Aarhus, Aarhus, DK-8000, Den.

Electrophoresis (1997), 18(3-4), 563-567

CODEN: ELCTDN; ISSN: 0173-0835

PUBLISHER:

SOURCE:

VCH Journal DOCUMENT TYPE:

LANGUAGE:

English

Chlamydia trachomatis is an obligate intracellular bacterium, inducing its AΒ own uptake in nonprofessional phagocytes either by phagocytosis or pinocytosis. We have previously shown that C. trachomatis L2 induces tyrosine phosphorylation of eukaryotic proteins upon their entry by phagocytosis. In this paper we characterize the tyrosine-phosphorylated proteins by two-dimensional gel electrophoresis. In immunoblotting with anti-phosphotyrosine antibodies of C. trachomatis L2-infected HeLa cells, but not with uninfected cells, two rows of spots were obsd. with a mol. mass of 69 and 71 kDa and pI from 5.0 to 5.2. In addn., a single spot of 100 kDa and pI 6.2 was obsd.

ANSWER 27 OF 54 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER:

1997:123519 HCAPLUS

Christiansen, Gunna

DOCUMENT NUMBER:

126:250047

TITLE:

The Mycoplasma hominis P120 membrane protein contains

a 216 amino acid hypervariable domain that is recognized by the human humoral immune response

Nyvold, Charlotte; Birkelund, Svend;

AUTHOR(S):

CORPORATE SOURCE:

Department of Medical Microbiology and Immunology,

University of Aarhus, Aarhus C, DK-8000, Den.

SOURCE:

Microbiology (Reading, U. K.) (1997), 143(2), 675-688

CODEN: MROBEO; ISSN: 1350-0872

PUBLISHER:

Society for General Microbiology

DOCUMENT TYPE:

Journal

LANGUAGE:

English

In the antigenically heterogeneous species Mycoplasma hominis a monoclonal antibody, mAb 26.7D, was previously found to recognize a 120 kDa polypeptide from M. hominis 7488. This antibody did not react with the type strain PG21. The homologous gene from M. hominis PG21 was cloned and sequenced and found to have a sequence identity of 91% with the gene of

strain 7488. One hypervariable and two semivariable regions were detected. The epitope for mAb 26.7D was mapped to the hypervariable domain by expression of various parts of this domain in Escherichia coli using expression vector systems. A polyclonal antiserum (pAb 121) generated against the hypervariable region of P120 from PG21 identified the P120 homolog in M. hominis PG21. Fusion proteins of the hypervariable and const. parts of the proteins were constructed and tested for reactivity with 21 human sera. Twelve sera reacted with the 7488 hypervariable fusion protein, but only four reacted with the PG21 hypervariable fusion protein. No reactivity was seen with a fusion protein contg. part of the const. region of P120. Gene fragments amplified from 18 M. hominis isolates by PCR confirmed the heterogeneity of the hypervariable domain. Based on restriction endonuclease cleavage patterns of the hypervariable domain the 18 isolates could be divided into four classes. Reactivity with both mAb 26.7D and pAb 121 confirmed these classes. The hypervariable, but not the const., part of P120 was recognized by the human humoral immune response. Such a variable domain may be important in evasion of the host's immune response, and thus aid survival of the micro-organism.

L6 ANSWER 28 OF 54 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1996:295775 HCAPLUS

DOCUMENT NUMBER: 125:2573

TITLE: Analysis of 0.5-kilobase-pair repeats in the

Mycoplasma hominis lmp gene system and identification

of gene products

AUTHOR(S): Ladefoged, Soeren A.; Jensen, Lise Torp; Brock,

Birgitte; Birkelund, Svend;

Christiansen, Gunna

CORPORATE SOURCE: Univ. Aarhus, Aarhus, Den.

SOURCE: J. Bacteriol. (1996), 178(10), 2775-2784

CODEN: JOBAAY; ISSN: 0021-9193

DOCUMENT TYPE: Journal

English LANGUAGE: Mycoplasma hominis, an opportunistic pathogenic bacterium of humans, has a AB small genome of 700 kb. Despite this, multiple copies of gene sequences with similarities to the structural gene (lmp1) of a 135-kDa surface-located membrane protein (Lmp1) have been identified on the genome of M. hominis PG21 (lmp2, lmp3, and lmp4). The distance between the lmp1-lmp2 region and the lmp3-lmp4 region was more than 110 kb. lmp3-lmp4of M. hominis PG21 was sequenced and found to contain two putative genes. The gene region of 6.5 kb contained a 5' unique region and a 3' unique region sepd. by 9 0.5-kb repeats with 51 to 90% similarity to 10 similar repeats found in the lmp1-lmp2 region. The 0.5-kb DNA repeats thus comprised about 1% of the entire genome. In both regions, a base change in one of the repeats gave rise to a stop codon, and thereby lmp2 and lmp4 By PCR amplification of reverse-transcriptase-generated cDNA it was shown that all four genes were transcribed. By use of Lmp-specific antibodies we showed that both lmp1 and lmp3 were translated into proteins (Lmp1 and Lmp3). Each of the four lmp genes represented by their unique cloned segments was used as a probe to analyze the presence, distribution, and organization of the genes within the genome in 13 M. hominis isolates. The repetitive element was detected at one or two locations on the chromosome for all isolates. The lmp3-specific element was present in all

09/446,677 Page 21 Shah

> isolates, and lmpl- and lmp2-specific elements were present in all but one isolate. The lmp4-specific element was present in about half the isolates tested. For five M. hominis isolates the chromosomal location of the lmp genes was mapped.

ANSWER 29 OF 54 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1996:286496 HCAPLUS

125:4975 DOCUMENT NUMBER:

Purification of recombinant Chlamydia trachomatis TITLE:

histone H1-like protein Hc2, and comparative

functional analysis of Hc2 and Hc1

Pedersen, Lotte Bang; Birkelund, Svend; AUTHOR(S):

Christiansen, Gunna

Dep. of Medical Microbiology and Immunology, CORPORATE SOURCE:

University of Aarhus, Aarhus C, DK-8000, Den.

Mol. Microbiol. (1996), 20(2), 295-311 SOURCE:

CODEN: MOMIEE; ISSN: 0950-382X

Journal DOCUMENT TYPE: English LANGUAGE:

The metabolically inactive developmental form of Chlamydia trachomatis, AΒ the elementary body, contains two very basic DNA-binding proteins with homol. to eukaryotic histone H1. One of these, Hc1, is relatively well characterized and induces DNA condensation in vitro, whereas the other, Hc2, is functionally virtually uncharacterized. In this study we describe the purifn. of Hc2, and a detailed comparative functional anal. of Hc2 and Hcl is presented. By gel shift assays and electron microscopy, marked differences in the nucleic acid-binding properties of Hc2 and Hc1 were obsd. Furthermore, Hc2 was found to strongly inhibit translation and transcription in vitro. Our results imply that DNA condensation is not the only function of Hc2.

ANSWER 30 OF 54 HCAPLUS COPYRIGHT 2001 ACS

1996:104880 HCAPLUS ACCESSION NUMBER:

124:225579 DOCUMENT NUMBER:

Mapping of Chlamydia trachomatis proteins by TITLE:

Immobiline-polyacrylamide two-dimensional

electrophoresis: spot identification by N-terminal

sequencing and immunoblotting

Bini, Luca; Sanchez-Campillo, Maria; Santucci, AUTHOR(S):

Annalisa; Magi, Barbara; Marzocchi, Barbara; Comanducci, Maurizio; Christiansen, Gunna; Birkelund, Svend; Cevenini, Roberto; et al. Dep. Mol. Biol., Siena Univ., Siena, Italy

CORPORATE SOURCE: Electrophoresis (1996), 17(1), 185-90

SOURCE:

CODEN: ELCTDN; ISSN: 0173-0835

DOCUMENT TYPE: Journal English LANGUAGE:

Proteins from purified elementary bodies of C. trachomatis were sepd. by 2-dimensional gel electrophoresis on nonlinear wide-range immobilized pH gradients in the first dimension and polyacrylamide gradient gels in the second dimension. The maps obtained with this system are highly reproducible and resolve .apprx.600 spots. By using immunoblot anal. with specific antibodies and/or N-terminal amino acid sequencing, the authors established the map positions of a no. of described chlamydial proteins,

Shah

such as the major outer membrane protein (MOMP) the 60 kDa cysteine-rich outer membrane protein (OMP2), the DnaK-like, GroEL-like, and macrophage infectivity potentiator (MIP)-like proteins, the plasmid-encoded pgp3 protein, two ribosomal proteins (S1 and L7/L12), and the protein-elongation factor EF-Tu. Other proteins, for which gene assignment was not possible, were identified by 3 parameters (Mr, pI and N-terminal sequence). This work provides a preliminary basis for a future and progressive compilation of a genome-linked database of chlamydial proteins.

L6 ANSWER 31 OF 54 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER:

1996:98222 HCAPLUS

DOCUMENT NUMBER:

124:139108

TITLE:

The 18-kilodalton Chlamydia trachomatis histone

H1-like protein (Hc1) contains a potential N-terminal

dimerization site and a C-terminal nucleic

acid-binding domain

AUTHOR(S):

Pedersen, Lotte Bang; Birkelund, Svend; Holm, Arne; Ostergaard, Soren; Christiansen,

Gunna

CORPORATE SOURCE:

Department Medical Microbiology, University Aarhus,

DK-8000, Den.

SOURCE:

J. Bacteriol. (1996), 178(4), 994-1002

CODEN: JOBAAY; ISSN: 0021-9193

DOCUMENT TYPE:

Journal

LANGUAGE:

English

The Chlamydia trachomatis histone H1-like protein (Hc1) is a DNA-binding protein specific for the metabolically inactive chlamydial developmental form, the elementary body. Hcl induces DNA condensation in Escherichia coli and is a strong inhibitor of transcription and translation. These effects may, in part, be due to Hcl-mediated alterations of DNA topol. To locate putative functional domains within Hc1, polypeptides Hc12-57 and Hc153-125, corresponding to the N- and C-terminal parts of Hc1, resp., were generated. By chem. crosslinking with ethylene glycol-bis(succinic acid N-hydroxysuccinimide ester), purified recombinant Hcl was found to form dimers. The dimerization site was located in the N-terminal part of Hc1 (Hc12-57). Moreover, CD measurements indicated an overall .alpha.-helical structure of this region. By using limited proteolysis, Southwestern blotting, and gel retardation assays, Hc153-225 was shown to contain a domain capable of binding both DNA and RNA. Under the same conditions, Hc12-57 had no nucleic acid-binding activity. Electron microscopy of Hcl-DNA and Hcl53-125-DNA complexes revealed differences suggesting that the N-terminal part of Hcl may affect the DNA-binding properties of Hcl.

L6 ANSWER 32 OF 54 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER:

1995:781405 HCAPLUS

DOCUMENT NUMBER:

123:193481

TITLE:

Selection of Mycoplasma hominis PG21 deletion mutants by cultivation in the presence of monoclonal antibody

552

AUTHOR(S):

Jensen, Lise Torp; Ladefoged, Soren; Birkelund,

Svend; Christiansen, Gunna

CORPORATE SOURCE:

Dep. Medical Microbiology and Immunology, Univ.

M. Smith 308-3278

Shah 09/446,677 Page 23

Aarhus, Aarhus, DK-8000, Den.

SOURCE: Infect. Immun. (1995), 63(9), 3336-47

CODEN: INFIBR; ISSN: 0019-9567

DOCUMENT TYPE: Journal LANGUAGE: English

Three mutants of Mycoplasma hominis PG21 were isolated and shown to AB contain alterations in the size of a repeat-contg. gene encoding a surface-localized 135-kDa antigen designated Lmp1. The mutants were isolated by cultivating M. hominis for a 3-mo period in the presence of Lmp1-specific monoclonal antibody (MAb) 552. The epitope for MAb 552 was localized at the repeated part of the protein. The gene encoding Lmpl is part of a transcriptional complex that contains 9.5 direct repeats of 471 bp each. Pure cultures of mutant strains were obtained by subcloning, and three mutants were characterized. The mutants showed deletions of a various no. of repeats. The deletions were accompanied by a decrease in size of the proteins. With increasing size of deletions, agglutination and growth inhibition by MAb 552 became less pronounced. Spontaneous aggregation of the mutant M. hominis cells in culture medium was, however, increased, indicating that the repeated elements may be of importance for repulsion of the cells.

L6 ANSWER 33 OF 54 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1995:558162 HCAPLUS

DOCUMENT NUMBER: 123:103656

TITLE: Semi-nested polymerase chain reaction for detection of

Pneumocystis carinii: implications for diagnosis,

prevalence and predictive parameters

AUTHOR(S): Oestergaard, L.; Tarp, B.; Jensen, B. Nybo; Henriques,

U.; Birkelund, S.; Christiansen, G.

; Andersen, P. L.

CORPORATE SOURCE: Department of Infectious Diseases, Marselisborg

Hospital, Aarhus, DK-8000, Den.

SOURCE: Immunol. Infect. Dis. (1995), 5(1), 59-66

CODEN: IINDEK; ISSN: 0959-4957

DOCUMENT TYPE: Journal LANGUAGE: English

The development of a semi-nested polymerase chain reaction (PCR) that provides semi-quantified results for detection of Pneumocystis carinii is described. The PCR was evaluated on bronchoalveolar lavage fluid and serum samples from HIV-infected patients with pulmonary impairment. When bronchoalveolar lavage fluid from 48 patients was examd., a prevalence of P. carinii pneumonia of 37.5% was found. The sensitivity and specificity of PCR were 94% and 97%, resp. Gomori methenamine silver staining showed a sensitivity and specificity of 59% and 100%, resp., and cytol. examn. consisting of Papanicolaou's and the May-Grunwald-Giemsa showed a sensitivity of 72% and a specificity of 100%. PCR did not detect P. carinii-DNA in serum obtained prior to clin. onset of P. carinii pneumonia. One of 7 serum samples obtained at the time of clin. P. carinii pneumonia was pos. by PCR. P. carinii pneumonia was the leading cause of pulmonary impairment both in patients receiving prophylactic antibiotic treatment against P. carinii and in patients not receiving prophylactic treatment. Toxoplasma gondii DNA was found by use of a nested PCR in bronchoalveolar lavage fluid from 3 patients. Cytomegalovirus (CMV) was isolated by quantitated culture in 66% of the

Shah 09/446,677 Page 24

cases and the clin. course was not affected by positivity of CMV.

L6 ANSWER 34 OF 54 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1995:279752 HCAPLUS

DOCUMENT NUMBER: 123:104016

TITLE: A 135-kilodalton surface antigen of Mycoplasma hominis

PG21 contains multiple directly repeated sequences

AUTHOR(S): Ladefoged, Soren A.; Birkelund, Svend;

Hauge, Steen; Brock, Birgitte; Jensen, Lise Torp;

Christiansen, Guanna

CORPORATE SOURCE: Dep. Med. Microbiol. Immunol., Univ. Aarhus, Aarhus,

DK-8000, Den.

SOURCE: Infect. Immun. (1995), 63(1), 212-23

CODEN: INFIBR; ISSN: 0019-9567

DOCUMENT TYPE: Journal LANGUAGE: English

A monoclonal antibody was used to characterize a 135-kDa surface-located AB membrane protein (Lmp1) generally present in Mycoplasma hominis strains. The monoclonal antibody, 552, was applied to identify the corresponding gene in an expression library of M. hominis PG21 DNA. The M. hominis PG21 lmp1 gene was sequenced, and its gene product was characterized with the goal of elucidating the structure and function of Lmpl. A total of 7196 bp in the lmp1 region was sequenced. An open reading frame of 4032 bp, encoding a protein of 1344 amino acids with a calcd. mol. wt. of 147,000, was identified. Anal. of the deduced amino acid sequence predicted a hydrophilic protein with a basic pI (10.0). The N-terminal 24 amino acids were a typical leader sequence. Downstream from the first 726 nucleotides, 6 similar direct repeats of 471 nucleotides were found. In repeat 7, a single-base substitution, C.fwdarw.A, gave rise to the stop codon of lmp1. Thus, the C-terminal 945 amino acids were encoded by the 471-bp direct repeats. As evidenced by Southern blot anal., the gene encoding the 135-kDa an igen is part of a multigene family. One of the genes, lmp2, was situated directly downstream from lmp1 where the direct repeats continued.

L6 ANSWER 35 OF 54 HCAPLUS COPYRIGHT 2001 ACS

ACCESSION NUMBER: 1995:33105 HCAPLUS

DOCUMENT NUMBER: 122:26123

TITLE: Analysis of a Mycoplasma hominis membrane protein,

P12(

AUTHOR(S): Christiansen, Gunna; Mathiesen, Soren L.;

Nyvold, Charlotte; Birkelund, Svend

CORPORATE SOURCE: Institute of Medical Microbiology, The Bartholin

Building, University of Aarhus, Aarhus-C, DK-8000,

Den.

SOURCE: FEM: Microbiol. Lett. (1994), 121(1), 121-8

COD: :: FMLED7; ISSN: 0378-1097

DOCUMENT TYPE: Journal LANGUAGE: English

AB The monoclonal antibody. Ab 26.7D generated against a clin. isolate of Mycoplasma hominis 7488 was shown to react with a surface-exposed epitope on a 120-kDa protein (P'''). The gene encoding the protein was cloned and sequenced, and the trap printional start point was detd, by primer

sequenced, and the tran priptional start point was detd. by primer extension anal. The geten contained an open reading frame of 3237 bp

encoding a peptide of 1079 amino acids with a deduced mol. mass of 123 kDa. A putative amino-terminal signal peptide and cleavage site for signal peptidase II were found. This suggests that the protein was synthesized as a precursor with subsequent processing to a mature lipoprotein. Surface emposure was confirmed by immunoelectron microscopy. Antibody mAb 26.7D reached with 11 of 19 M. hominis strains. The gene was, however, present in all strains.

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ANSWER 36 OF 54 HCAPLUS COPYRIGHT 2001 ACS
ACCESSION NUMBER:
                           199°:18529 HCAPLUS
DOCUMENT NUMBER:
                           122:37685
                           Development of a nested polymerase chain reaction
TITLE:
                           usin; time-resolved fluorometry for automated
                           det :tion of Chlamydia trachomatis
                           Oes rgaard, L.; Moeller, J. K.; Birkelund, S.
AUTHOR(S):
                           ; Chaistiansen, G.; Andersen, P. L.
CORPORATE SOURCE:
                           Mar elisborg Hosp., Aarhus Univ. Hosp., Aarhus,
                           DK- 000, Den.
                           Imm nol. Infect. Dis. (1994), 4(1), 36-40
SOURCE:
                           CODER: IINDEK; ISSN: 0959-4957
DOCUMENT TYPE:
                           Journal
                           Eng'sh
LANGUAGE:
     The polymerase chain rection (PCR) has proven successful for detection of
AΒ
     Chlamydia trachomatis.
                                 .e development of a nested PCR for automated
     detection of amplified ( . trachomatis DNA by use of time resolved
     fluorometry is described. The system was capable of detecting an amt. of DNA corresponding to less than one C. trachomatis genome when either
     purified C. trachomatis THA or elementary bodies were used as target DNA. A correlation between the amt. of fluorescence measured and the no. of C.
     trachomatis genomes was ween. Of 109 patient samples evaluated, seven
     were pos. by both culti
                                 and PCR. A hundred samples were neg. by both
     methods. Two samples w: pos. by PCR and neg. by culture. These two samples were from patie: who either had, or might have had, chlamydial
     infection. A clear distanction between pos. and neg. samples was obsd.
     ANSWER 37 OF 54 HCAPLU. COPYRIGHT 2001 ACS
ACCESSION NUMBER:
                           199 : 578371 HCAPLUS
                           12:
DOCUMENT NUMBER:
                                 13371
                           Ch
                                  terization of a linear epitope on Chlamydia
TITLE:
                                  matis serovar L2 DnaK-like protein
                           tra
AUTHOR(S):
                           Bir.
                                  und, Svend; Larsen, Bente; Holm, Arne;
                           Lun
                                 ose, Anker G.; Christiansen, Gunna
                           Ins Med. Microbiol., Univ. Aarhus, Aarhus, DK-8000,
CORPORATE SOURCE:
                           In 10^{-10}. Immun. (1994), 62(5), 2051-7
SOURCE:
                                 : INFIBR; ISSN: 0019-9567
                           COL
DOCUMENT TYPE:
                           Jo.
                                  1
LANGUAGE:
                           En
                                  ogen from Chlamydia trachomatis serovar L2 has
AB
     A cytoplasmic 75-kDa in
                                  ed as being similar to the Escherichia coli
     previously been charact
     heat shock protein Dnall
                                  'e have localized a linear epitope for one
     monoclonal antibody specific for C. trachomatis DnaK. By use of a
     recombinant DNA techni . the epitope was limited to 14 amino acids.
     With synthetic peptide: he epitope was further limited to eight amino
```

Shah 09/446,677 Page 26

acids. Six of these amino acids are conserved in bovine HSP70, which has a known three-dimensional structure. The amino acid sequence homologous to the epitope is located in a linear part of the HSP70 mol. known as connect II.

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ANSWER 38 OF 54 HCAPLUS COPYRIGHT 2001 ACS
1.6
                         1994:077861 HCAPLUS
ACCESSION NUMBER:
                         121:277861
DOCUMENT NUMBER:
                         Chlamidia trachomatis serovar L2 induces protein
TITLE:
                         tyre the phosphorylation during uptake by HeLa cells
AUTHOR(S):
                         Birk Jund, Svend; Johnsen, Helle;
                         Chri iansen, Gunna
                         Inst. Med. Microbiol., Univ. Aarhus, Aarhus, DK-8000,
CORPORATE SOURCE:
                         Den.
                         In: - Immun. (1994), 62(11), 4900-8
SOURCE:
                         COL: 1: INFIBR; ISSN: 0019-9567
                         Jour al
DOCUMENT TYPE:
LANGUAGE:
                         Engl h
                               s an obligate intracellular microorganism with a
     Chlamydia trachomatis 1
AB
     unique biphasic life c
                                  The extracellular form, the elementary body
     (EB), is infectious but: "abolically inactive. Attachment of EBs to host
     cells is mediated by a harmonic ran sulfate-like glycosaminoglycan. Following
     attachment, the EB is in analized within a membrane-bound vesicle, and
     during the first 8 h of
                               fection the vesicles are transported to a
     perinuclear location with they aggregate and fuse. By use of a
     monoclonal antibody against phosphotyrosine, the authors showed that three
                               rosine phosphorylated: a triple band of 68, 66,
     classes of proteins ar-
                                and a 140-kDa band. The phosphorylation could
     and 64 kDa, a 97-kDa b
                               ing from 15 min after infection of HeLa cells.
     be detected by immunoble
     The authors followed the
                                vement of the EBs and the tyrosine
     phosphorylation of pro--
                               by double-labeling immunofluorescence
     microscopy with the same
                               moclonal anti-phosphotyrosine antibody and a
     polyclonal antibody ag
                               the C: trachomatis L2 outer membrane complex.
                              infection, the phosphorylation colocalized with
     During the first 8 h o
                             infection, EBs have reorganized to the
     EBs. Sixteen hours af
                              es, forming an inclusion. At this time,
     replicating reticulate
                              : dotted spots in the periphery of the
     phosphorylation was se-
     inclusion.
    ANSWER 39 OF 54 HCAPL
                                OPYRIGHT 2001 ACS
                         19 : ::
                                1694 HCAPLUS
ACCESSION NUMBER:
                         12
DOCUMENT NUMBER:
                                694
                                terization of a Mycoplasma hominis gene encoding
                         Ch.
TITLE:
                         1 y
                                .RNA synthetase (LysRS)
                                n, Derya; Birkelund, Svend;
AUTHOR(S):
                         0::
                                ansen, Gunna
                         Ch.
CORPORATE SOURCE:
                         INs:
                                [ed. Microbiol., Univ. Aarhus, Aarhus, DK-8000,
                         Don.
                         FE: 1 crobiol. Lett. (1994), 116(3), 277-82
SOURCE:
                         CC 11
                                FMLED7; ISSN: 0378-1097
DOCUMENT TYPE:
                         Jc:
LANGUAGE:
                         Ei
                                'A synthetase (lysS) in Mycoplasma hominis was
     The gene encoding lys:
                                gene has an open reading frame of 1466\ \mathrm{bp}
     cloned and sequenced.
```

Page 27

encoding a polypeptide with a predicted mol. mass of 57 kDa. The amino acid sequence showed 44.5 and 43.7% identity to the Escherichia colilysyl-tRNA synthetases, encoded by lysS and lysU. Only one lysyl-tRNA synthetase encoding gene was found in M. hominis. The G + C content of the gene was 28.6%, which is significantly lower than in other prokaryotes. The gene was located 4 kb upstream of the M. hominis PG21 rRNA B operon.

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ANSWER 40 OF 54 HCAPLUS COPYRIGHT 2001 ACS
                         199 at a 7163 HCAPLUS
ACCESSION NUMBER:
                         126:1
                               163
DOCUMENT NUMBER:
                         Interaction of the Chlamydia trachomatis histone
TITLE:
                         Hl-... protein (Hcl) with DNA and RNA causes
                         repression of transcription and translation in vitro
                               en, Lotte Bang; Birkelund, Svend;
                         Peri
AUTHOR(S):
                         Chri iansen, Gunna
                         Inst. Med. Microbiol., Univ. Aarhus, Aarhus, DK-8000,
CORPORATE SOURCE:
                         De:.
                                crobiol. (1994), 11(6), 1085-98
                         Mc . .
SOURCE:
                         CC ! , ' ' '
                                MOMIEE; ISSN: 0950-382X
                         Jc∷
DOCUMENT TYPE:
                         Enc :
LANGUAGE:
                                protein from Chlamydia trachomatis (Hcl) is a
     The 18 kDa histone H1-
AB
                               . to be involved in condensation of the
     DNA-binding protein that
                               g late stages in the chlamydial life cycle.
     chlamydial chromosome
                                ichia coli results in an overall relaxation of
     Expression of Hcl in i
                                A, RNA and protein synthesis. The authors have
     DNA and severely affec
                                 Hcl with single-stranded DNA and RNA by
     analyzed the interaction:
                                 on blotting. Furthermore, the authors show
     Southwestern and north
                                Hcl dramatically affects transcription and
     that purified, recombin
                                "siol. relevant concns. These results were
     translation in vitro a
                                 formation of condensed Hcl-DNA and Hcl-RNA
     found to coincide with
                                prose gel electrophoresis and electron
     complexes as revealed
                                n of these results for possible functions of
     microscopy. The impl:
     Hcl in vivo are discu-
     ANSWER 41 OF 54 HCAPI
                                TOPYRIGHT 2001 ACS
                                667 HCAPLUS
ACCESSION NUMBER:
                         19:
                                ...567
                         114
DOCUMENT NUMBER:
                                columerase chain reaction for detection of
                         Usa
TITLE:
                                ia trachomatis. [Erratum to document cited in
                         C^{1}_{-1}
                         C7.
                                 1:55202z]
                                 hard, Lars; Birkelund, Svend;
                         0
AUTHOR(S):
                                 ansen, Gunna
                         C.
                                 d. Microbiol., Univ. Aarhus, Aarhus, DK-8000,
                         In:
CORPORATE SOURCE:
                         D_{\cdot}
                                 . Microbiol. (1993), 31(11), 3081
                         J.
SOURCE:
                         \mathbf{C}^{r} :
                                 'CMIDW; ISSN: 0095-1137
                          J.
DOCUMENT TYPE:
                         Er
LANGUAGE:
                                 ed in the abstr. or the index entries.
ΑB
     The errors were not re
                                 PYRIGHT 2001 ACS
     ANSWER 42 OF 54 HCAPI
                                 562 HCAPLUS
                         1
ACCESSION NUMBER:
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DOCUMENT NUMBER: 119: 562 Chla yaia trachomatis Mip-like protein TITLE: Lun ...se, Anker G.; Rouch, Duncan A.; Birkelund, AUTHOR(S): · Christiansen, Gunaa; Pearce, $\mathbf{s}_{\mathbf{v}}$. Jc! Med. Microbiol., Univ. Aarhus, Aarhus, DK-8000, Inch CORPORATE SOURCE: De:. Mol. 'icrobiol. (1992), 6(17), 2539-48 SOURCE: CC: . MOMIEE; ISSN: 0950-382X Jour DOCUMENT TYPE: En LANGUAGE: tis Mip-like protein with homol. to a A 27 kDa Chlamydia tr AB ragment of the surface-exposed Legionella 175-amino-acid C-term: t has previously been described. In this paper pneumophila mip-gene ke sequence of C. trachomatis serovar L2 the entire chlamydia 1. LGV) biovar] is presented. The sequence shows [lymphogranuloma vene: onella Mip protein and its C-terminal region, high similarity to the Mip, has high amino acid similarity to like that of the legio: FK506-binding proteins. The chlamydial eukaryotic and prokar by polymerase chain reaction (PCR) in other C. mip-like gene was def sequencing of the mip-like genes of serovars B trachomatis serovars : shown to be highly conserved within the two and E (trachoma biova: matis. Monoclonal and polyclonal antibodies majors biovars of C. ant Mip-like protein failed to demonstrate raised against the recinfectious elementary bodies or reproductive surface-exposed epito by immunofluorescence or immuno-gold electron reticulate body forms "lement-dependent inhibition of up to 91% of microscopy. However, s was obsd. with antibodies to the N-terminal infectivity for cell tein suggesting that antibody-accessible fragment of the Mip-1 .ectious EBs. epitopes are present ANSWER 43 OF 54 HCAP OPYRIGHT 2001 ACS ACCESSION NUMBER: 1 3014 HCAPLUS DOCUMENT NUMBER: 7 ٦14 mion between the Chlamydia trachomatis histone TITLE: 1.. protein (Hcl) and DNA H nsen, Gunna; Pedersen, Lotte Bang; C AUTHOR(S): Jane E.; Lundemose, Anker G.; Birkelund, ŀ. 'ed. Microbiol., Univ. Aarhus, Aarhus, DK-8000, CORPORATE SOURCE: l. eriol. (1993), 175(6), 1785-95 J SOURCE: OBAAY; ISSN: 0021-9193 C DOCUMENT TYPE: LANGUAGE: chomatis Hcl from serovar L2 was cloned into The gene encoding the AB mpression vector pET11d. In this vector, Escherichia coli by s under the control of a bacteriophage T7 transcription of the rase is inducible in the host. Following promoter, and T7 RNA s were lysed gently. Gel filtration of the induction, the E. col of DNA and Hcl in the voided vol. Electron lysate revealed comic to be complexed with protein in large microscopy revealed t of spherical bodies. Purified recombinant

ng capacity and was able at high concns. to

aggregates, often in Hcl maintained its D:

09/446,677

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P 28

29

TBASE2

Shah

SOURCE:

form condensed aggregat independently of the I for supercoiled DNA. spherical bodies.

with DNA (one mol. of Hcl per base pair) or size of the DNA but with a slight preference alone is thus able to package DNA into condensed

ANSWER 44 OF 54 HCAPL' 76 ACCESSION NUMBER: 11 DOCUMENT NUMBER: Use TITLE: 7. L_{ϵ} AUTHOR(S): Bi. Ιn· CORPORATE SOURCE: 700

COPYRIGHT 2001 ACS 5623 HCAPLUS 62**3** monoclonal antibodies for detection of antigen on in Mycoplasma hominis ed, Soren; Hauge, Steen; Andersen, Hans; and, Svend; Christiansen, Gunna

IV. C DOCUMENT TYPE:

!ed. Microbiol., Univ. Aarhus, Aarhus, Den. bl. Bakteriol., Suppl. (1990), 20 (Recent Adv. smol.), 634-9

LANGUAGE: AB The M. hominis is a h the female genital tr involved in acute pel: postpartum fever. In twelve monoclonal ant The MAb were classifi By immunofluorescence epitopes were cytopla proteins. Three MAb were all conserved in surface exposed prote integral membrane pro: the surface exposed I while the remaining ! strains.

enous group of mycoplasmas commonly found in It is potentially pathogenic and may be flammatory disease, acute pyelonephritis and to analyze antigen variation in M. hominis, 5 (MAb) against M. hominis PG21 were produced. their epitopes in M. hominis PG21 were detd. citon-X-114 sepn. it was detd. whether the :urface localized or integral membrane t with cytoplasmic antigens and their epitopes Her M. hominis strains. Nine MAb reacted with d all of these except one were shown to be By immunoblotting it was shown that only 3 of cted with all the 25 other M. hominis strains sted with between 6 and 24 other M. hominis

ANSWER 45 OF 54 HCAT ACCESSION NUMBER: DOCUMENT NUMBER: TITLE:

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ŀ 1:

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PYRIGHT 2001 ACS 560 HCAPLUS

AUTHOR(S):

SOURCE:

J60 monoclonal antibodies for detection of gene and variation in Mycoplasma hominis nsen, Gunna; Ladefoged, Soeren; teen; Birkelund, Svend; Andersen,

CORPORATE SOURCE:

d. Microbiol., Univ. Aarhus, Aarhus, Den. 1. Bakteriol., Suppl. (1990), 20(Recent Adv. mol.), 535-45 :BASE2

DOCUMENT TYPE: LANGUAGE:

(MAb) against surface exposed M. hominis PG21 d their epitopes in M. hominis PG21 were 114 sepn. it was detd. whether the epitopes ins. These MAb were used in immunoblotting to genic epitope in 25 other M. hominis strains. strains while the 3rd reacted only with 12

Three monoclonal anti antigens were classi: characterized. By T were integral membra show the presence of Two MAb reacted with :

Lu

1.

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strains and in some sty PG21 antigen. The MA Sau3A contg. cleaved pEX1, 2 and 3. Using Southern blotting anal. hominis strains was ushybridization patterns variation in immunoble:

: with polypeptides different in size from the : used to screen for recombinant E. coli contg. MA fragments ligated to the expression vectors id DNA from the recombinant clones as probes, restriction enzyme cleaved DNA from 26 M. characterization; a variation in the scribed. This variation was compared to the results.

ANSWER 46 OF 54 HCAP ACCESSION NUMBER: 5. DOCUMENT NUMBER: (TITLE: į. TOPYRIGHT 2001 ACS 38 HCAPLUS

AUTHOR(S):

trachomatis contains a protein similar to onella pneumophila mip gene products A. G.; Birkelund, S.; Fey, S. J.;

CORPORATE SOURCE:

sen, P.; Christiansen, G. d. Microbiol., Univ. Aarhus, Aarhus, DK-8000,

SOURCE:

mobiol. (1991), 5(1), 109-15 MIEE; ISSN: 0950-382X

DOCUMENT TYPE: LANGUAGE:

 $_{\scriptscriptstyle \rm E}$ rotein was characterized by the use of

A 27 kDa C. trachoma: AB monoclonal antibodie: protein was shown to well as elementary bo post-infection. Clo revealed an open rea deduced amino acid s homol. between the 2 the macrophage infec

v two-dimensional gel electrophoresis. The med in the membrane of reticulate bodies as lts synthesis could be detected from 10 h : sequence anal. of the distal part of the gene of 175 amino acids. Comparison of the ith the NBRF data base revealed significant amydial membrane protein and the product of tentiator (mip) gene of L. pneumophila.

ANSWER 47 OF 54 HCA 1.6 ACCESSION NUMBER: 1 DOCUMENT NUMBER: TITLE:

TRIGHT 2001 ACS 1 HCAPLUS

AUTHOR(S):

lodalton cytoplasmic Chlamydia trachomatis L2 le is a DnaK-like protein

, Svend; Lundemose, Anker G.;

CORPORATE SOURCE:

⊲en, Gunna Microbiol., Univ. Aarhus, Aarhus C, , ⊃en.

SOURCE:

anun. (1990), 58(7), 2098-104

FIBR; ISSN: 0019-9567

DOCUMENT TYPE: LANGUAGE:

The gene coding for : AB polypeptide has been sequence has been de region as well as the of the 1980-base-pai 75-kilodalton protei: DnaK proteins of E. homol. with human he region was identifie

lodalton cytoplasmic C. trachomatis L2 Escherichia coli, and the nucleotide loned DNA fragment contained the coding : promoter. The deduced amino acid sequence ding frame revealed 94% homol. with a trachomatis serovar D and 5% homol. with the of Bacillus megaterium, while amino acid protein 70 (hsp70) was 42%. The promoter ter search and by primer extension of mRNA

. coli. The promoter region which differed synthesized in recombiragion in serovar D was shown to be a mixed from the putative pro: promoter type in which ·:) region showed a regular TATA box configuration while the region showed high homol. with heat shock promoters. This mixed er was recognized in E. coli. ANSWER 48 OF 54 HCAPL YRIGHT 2001 ACS 38 HCAPLUS ACCESSION NUMBER: 19 1:3 DOCUMENT NUMBER: C^{\dagger} ization and identification of early proteins TITLE: 3 1 Hia trachomatis serovar L2 by two-dimensional ophoresis] Anker G.; Birkelund, Svend; AUTHOR(S): ster Mose; Fey, Stephen J.; 1 C', en, Gunna Microbiol., Univ. Aarhus, Aarhus, DK-8000, CORPORATE SOURCE: 1... mmun. (1990), 58(8), 2478-86 T:: SOURCE: IBR; ISSN: 0019-9567 DOCUMENT TYPE: LANGUAGE: The synthesis of ear' s from C. trachomatis serovar L2 was AΒ analyzed by two-dime l electrophoresis. By pulse-label expts., the synthesis of seve s was obsd. at 2 to 8 h postinfection before in was detected at 8 to 10 h after the major outer memb infection. The early : were synthesized throughout the 30-h period \circ of three proteins of 75, 62, and 45 investigated, but the kilodaltons decreasec to 30 h postinfection. Pulse-chase anal. showed that the signa e same three proteins declined 26 to 30 h after infection. The · early proteins were identified as the S1 ike protein, and DnaK-like protein, resp. ribosomal protein, t ANSWER 49 OF 54 HCA'. RIGHT 2001 ACS ACCESSION NUMBER: 2 HCAPLUS 11 DOCUMENT NUMBER: 1. wmerase chain reaction for detection of TITLE: (, rachomatis !, Lars; Birkelund, Svend; AUTHOR(S): n, Gunna Microbiol., Univ. Aarhus, Aarhus, DK-8000, CORPORATE SOURCE: icrobiol. (1990), 28(6), 1254-60 SOURCE: IDW; ISSN: 0095-1137 (DOCUMENT TYPE: J-LANGUAGE: ~R) assay was developed for detection of C. A polymerase chain reished sequence of the common C. trachomatis trachomatis DNA. Fro plasmid, two primer elected. Detection of amplified sequences phoresis of cleaved or uncleaved amplified was done by agarose sequences, Southern ! on, or dot blot anal. The PCR assay was optimized and, after of amplification with primer set II, 10-17 g DNA, which corresponds to the demonstrated a sensit detection of one copy lasmid. Because of the high sensitivity, a

which airborne contamination was minimized.

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closed system was deve.